Jour. Agric. Res. China (中華農業研究) 35(3)375~386(1986)

Insecticide Resistance Study in *Plutella xylostella* (L.)

VIII. The Specificity of Oxidative Detoxication Mechanism in Larval Stage[1]

Edward Y. Cheng, C. H. Kao, D. F. Lin and T. C. Tsai[2]

Abstract: In this study, we have observed that the resistance of DBM to organophosphorus insecticides are not associated with MFOs, and the natural declining rate of organophosphorus-resistance is closely correlated with the resistance level of different compounds in the field. Mevinphos, phenthoate, and methidathion are three organophosphorus insecticides with the fastest resistance declining rate, and also meet minimum resistance problem in the field On the other hand,methamidophos, mephosfolan, and diethquinalphion have the slowest rate and also met with enormous resistance in the field. Synthetic pyrethroids and carbamates are chiefly detoxicated by MFOs in DBM. By using separate DBM strains and with the help of piperionyl butoxide and other synergists, we have concluded that DBM can develop three separate MFOs for (1) synthetic pyrethroids, (2) carbofuran, and (3) carbaryl, propoxur, and methomyl. It seems that the cross resistance is not existed among three MFOs sub-systems. The evidences are present in Tables and discussed in the text.

Introduction

There are many insecticides has been used for controlling the diamondback moth, *Plutella xylostella* (L.), in Taiwan over the past 15 years. Recently we have found that some of these insecticides still retain good control efficacy despite the insects can generate high resistance to insecticides [18] rapidly. Mevinphos, phenthoate, and methidathion are belonged to this catagory of durable insecticides and they are all organophosphorus (OP) compounds. So far there is no explanation for it. The DBM usuallly generate higher resistance toward carbamates and synthetic pyrethroids (SP) than that for the OP-insecticides [7,11]. The mixed function oxidases (MFOs) are closely

1. Contribution No. 1295 from Taiwan Agricultural Research Institute. The study is supported by NSC research grant 75-0409-B055-10 and CAPD research grant 74-4.1-147 (03).

2. Senior entomologist, research assistant, NSC project assistant, and CAPD project assistant, respectively. Thanks due to the excellent laboratory assisting work of Ms Su-bay Jean and Yen-ling Lu. Department of Applied Zoology, TARI, Wufeng, Taichung, Taiwan 41301, Republic of China,

associated with the SP-resistance in DBM [19], but their relationship with other insecticide resistances has not been clarified yet. The MFOs are the main detoxication mechanism for pyrethrin [12,23] and carbamates [6,21], and they also involve in the activation/inactivation of OP insecticides *in vivo* [16,17].

In the western coast of Taiwan, the DBMs are highly resistant to both synthetic pyrethroids and carbamates except carbofuran [7,10,18]. However, low degree of carbofuran resistance is commonly existed and no correlation to other insecticide resistance has been found [18]. We had reported that the DBM from I-lan county was extremely sensitive to both carbofuran and synthetic pyrethroids but not to other carbamates [11]. Are the MFOs responsible for this ? Usually, the MFOs are treated as one entity with many common features; Agosin [2] had pointed out that: "the possibility of several MFOs in insects, instead of a single non-specific one"; Yu and Terriere [27,28] had confirmed the existence of multiple-forms cytochrome P-450, a constitute of MFOs, in several dipterous species. The MFO-diversity may exist in the DBM as well as in the dipterous species. Besides, the substrate-specificity of different MFOs in Ndealkylation, S-dealkylation, O-dealkylation, and aromatic ring-hydroxylation etc., also make the detailed analysis of MFOs action difficult. Whether the MFOs can cause cross resistance among different insecticides is the major interest in this investigation; although the minor cross effect seems unavoidable, only the serious cross resistance is the target for studying.

In the following study, we will compare: (1) the declining rate of resistance after the DBMs were relieved from insecticide-selection pressure, (2) the MFOs activity in relation to different insecticide resistances, with special emphasis on the substrate-specificity of MFOs toward different insecticides.

Materials and methods

Insecticides: compounds used in both the resistance decline study and the MFOs activity in relation to different insecticide resistances study were commercial grade mevinphos, profenofos, prothiofos, methidathion, mephosfolan, phenthoate, diethquinalphion, methamidophos, decamethrin, permethrin, fenvalerate, carbofuran, carbaryl, methomyl, and propoxur. Insecticides were diluted in water to proper concentrations for assay through the Burkard Potter spray tower. In the MFOs substrate-specificity study, purified carbaryl, methomyl, propoxur, and carbofuran were dissolved in acetone and applied topically on the insects by the microapplicator.

Synergists: piperionyl butoxide (pb) was obtained from Tokyo Kasei; MGK 264 was a gift from Cheng-Hong Chemical Co., Ltd.; and 4-chloro-1-naphthyl 2-propynyl ether (pe) was obtained from Union Carbide, Taiwan Ltd.

Testing procedures and insects:

1. The resistance decline study--the resistant DBM was collected from an intensively cultivated vegetable district near Taipei. The starting generation for testing was F_2 because the F_1 generation was solely used for propagating sufficient DBM. The susceptibi-

lities of DBM to 12 insecticides were tested on F_2, F_5, F_8 and F_{11}, or at 3-generations interval. Usually, 7 dosages from LC_{10} to LC_{95} were made in distilled water for testing. Each dosage was tested on 40-60 third instar DBM larvae in three replicas. The testing procedure is the same spraying operation adapted in this laboratory[11]. The post-treatment holding temperature and relative humidity were controlled at 25±1°C and 85±10% respectively. The mortality of treated insects was counted at 24 hours after the spray, and all results were analyzed in probits[15].

2. The study of MFOs activity in relation to different insecticide resistances--the susceptible I-lan strain and the F_5 generation of Taipei resistant strain were used to test the role of oxidative detoxication toward different insecticides. Two commercial synergists, pb and MGK 264 were added into insecticides at 5 to 1 ratio then followed the spray-testing procedure as described in (1).

3. The MFO specificity test--in order to resolve the specificity of MFOs toward different carbamates, a highly resistant DBM strain from Taichung was used. Instead of the spraying method, the topical application method was adapted and the insec ticide/acetone solution with or without synergist were used in the treatment. The less effective MGK 264 was substituted by a stronger synergist, pe[3,14,25]. All changes are aimed at providing more direct and precise measurement in MFO's substrate-specificity. The third instar larvae were immobilized in a petri dish placed on ice. Each larva receive 0.5μl acetone solution of designated insecticide/synergist combination through the syringe from microapplicator. Seven dosages within the LD_{10} to LD_{95} of each insecticide or insecticide/synergist treatment were tested on 60 larvae in three replicas. All post-treatment handling were the same as that of (1).

Table 1. The susceptibility changes of resistant DBM to twelve commonly used insecticides after the insecticide pressure was relieved.

Insecticides	LC_{50} in ppm, (slope)				The lowest LC_{50} ever recorded in Taiwan (ppm)
	F_2	F_5	F_8	F_{11}	
Organophosphorus					
Mevinphos	537.1 (1.57)	232.9 (1.06)	161.7 (1.05)	220.1 (1.37)	73*
Profenofos	928.8 (1.28)	662.2 (1.11)	558.5 (0.99)	410.4 (1.05)	100*
Prothiofos	2,683.6 (1.44)	2,165.3 (0.93)	1,112.8 (1.16)	1,219.0 (1.45)	310*
Methidathion	776.1 (1.48)	464.9 (2.47)	322.4 (1.13)	490.3 (1.73)	270*
Mephosfolan	1,487.0 (0.97)	2,456.0 (0.82)	743.5 (0.82)	1,796.1 (0.91)	320**
Phenthoate	1,161.7 (1.37)	677.3 (1.19)	444.8 (0.75)	77.1 (1.34)	262***
Diethquinalphion	1,954.4 (1.24)	1,755.2 (0.92)	1,192.7 (0.95)	3,059.4 (0.86)	230*
Methamidophos	3,269.2 (1.44)	3,983.7 (0.91)	1,778.9 (1.23)	3,579.0 (1.34)	560*
Synthetic pyrethroids					
Decamethrin	656.2 (1.03)	214.7 (0.46)	65.2 (0.47)	92.9 (0.79)	4*
Fenvalerate	1,862.7 (1.76)	757.0 (0.61)	179.6 (0.77)	221.9 (0.93)	9*
Permethrin	1,148.8 (1.18)	353.2 (0.85)	64.0 (0.99)	43.61 (1.55)	14*
Carbamate					
Carbofuran	763.5 (1.25)	570.0 (0.67)	213.7 (0.38)	241.7 (0.74)	75***

*Reference 11.

**Recorded on DBMs of Su-ao of I-lan county in 1982. Unpublished information.

***Recorded on DBMs of Tou-cheng of I-lan county in 1982. Unpublished information.

Results

1. The resistance decline study: once the insecticide selection pressure was removed, the resistant DBM gradually relieved their resistance toward all three major groups of insecticides (Table 1). The lowest insecticide LC_{50}s ever recorded for DBM in Taiwan were also given. The data in Table 1 were then converted to resistance ratios (R. R.) for comparison, and their resistance declining rate were also calculated in Table 2. Compared to other insecticides, the synthetic pyrethroids had met with the strongest resistance as the R. R. ranged from 82.06 to 206.96; the ratios were 10.18 for carbofuran and 2.87-9.29 for eight OP compounds. The SP-resistance declined very rapidly, and the declining rates were extremely similar among three tested compounds; only one-tenth of original resistance remained after 6 insecticide-free generations for all three synthetic pyrethroids tested. Carbofuran resistance declined rather slowly in the beginning and then in a much faster pace which was next to that of SP-resistance, but still faster than the OP-resistance.

Table 2. The decrease of resistance to twelve commonly used insecticides of a resistant DBM strain after relieving from the insecticide pressure.

Insecticides	Resistance Ratio*				% Decreases			
	F_2	F_5	F_8	F_{11}	F_2	F_5	F_8	F_{11}
Organophosphorus								
Mevinphos	7.35	3.19	2.22	3.01	0.0	56.6	69.9	59.0
Profenofos	9.29	6.62	5.59	4.10	0.0	28.7	39.9	55.8
Prothiofos	8.66	6.98	3.59	3.93	0.0	19.3	58.5	54.6
Methidathion	2.87	1.72	1.19	1.82	0.0	40.1	58.5	36.6
Mephosfolan	4.65	7.68	2.32	5.61	0.0	0.0	50.0	0.0
Phenthoate	4.43	2.59	1.70	0.29	0.0	41.7	61.7	93.4
Diethquinalphion	8.50	7.63	5.19	13.30	0.0	10.2	39.0	0.0
Methamidophos	5.84	7.11	3.18	6.39	0.0	0.0	45.6	0.0
Synthetic pyrethroids								
Decamethrin	164.05	53.68	16.30	23.22	0.0	67.3	90.1	85.8
Fenvalerate	206.96	84.11	19.96	24.66	0.0	59.3	90.4	88.1
Permethrin	82.06	25.23	4.57	3.12	0.0	69.3	94.4	96.2
Carbamate								
Carbofuran	10.18	7.60	2.84	3.22	0.0	25.3	72.0	68.3

*Calculated from Table 1.

After 9 insecticide-free generations, the resistances of F_{11} to eight OP compounds had declined 0~93. 4% in the order of: phenthoate>mevinphos>profenofos>prothio-

fos>methidathion>mephosfolan>methamidophos>diethquinalphion. In F_5, the differences between compounds were already obvious. The reduction of resistance for mevinphos, phenthoate, and methidathion were 40-60%, while the resistance for mephosfolan, methamidophos, and diethquinalphion showed no reduction at all.

2. The study of MFOs activity in relation to different insecticide resistances: the responses of both susceptible and resistant DBM to three major catagories of insecticides and their mixtures with pb or MGK 264 were compared in Table 3, 4, and 5. No ill effect was noticed in insects treated with the synergists only at the test concentration. Both synergists exerted none or only minor synergistic effects on the OP insecticides in DBM (Table 3). For pb, the synergistic ratios varied from 0.76-2.74 and 1.04-2.25 for the susceptible and resistant DBM respectively. Meanwhile, MGK 264 did not induce different results from that of pb.

Table 3. The effects of piperionyl butoxide and MGK 264 on the DBM resistance to organophosphorus insecticides. (LC_{50} in ppm)

Insecticides	Original	+PB		+MGK 264	
	LC_{50} (slope)	LC_{50} (slope)	S. R.*	LC_{50} (slope)	S. R.*
Susceptible strain					
Mevinphos	90.8 (1.08)	37.7 (1.05)	2.41	106.3 (1.06)	0.85
Profenofos	367.0 (0.94)	134.1 (1.38)	2.74	237.6 (0.98)	1.54
Prothiofos	831.0 (0.79)	710.2 (0.70)	1.17	410.1 (0.55)	2.03
Methidathion	451.9 (0.87)	282.6 (0.87)	1.59	171.7 (0.94)	2.63
Phenthoate	283.9 (0.76)	371.4 (0.65)	0.76	347.9 (1.31)	0.82
Resistant strain					
Mevinphos	232.9 (1.06)	122.6 (1.13)	1.89	164.1 (0.97)	1.42
Profenofos	662.2 (1.11)	294.5 (1.82)	2.25	298.2 (0.62)	2.20
Prothiofos	2,165.3 (0.93)	1,526.7 (1.74)	1.42	901.9 (0.90)	2.40
Methidathion	464.8 (2.47)	447.0 (1.05)	1.04	445.2 (1.13)	1.04
Phenthoate	677.3 (1.19)	320.6 (1.01)	2.11	422.5 (0.86)	1.60

* S. R. =the synergistic ratio

On the contrary, the synergists resulted significant synergistic effects in the synthetic pyrethroids (Table 4). With the help of pb, the resistant DBM restored their SP-sensitivity as that of the susceptible DBM. MGK 264 is less effective than pb. In contrast to the lowest LC_{50} ever recorded in Taiwan, there is a minor portion of SP-resistance which was not overcome by the action of MFO inhibitor-type synergists.

Table 4. The effects of piperionyl butoxide and MGK 264 on the DBM resistance to synthetic pyrethroids. (LC_{50} in ppm)

Insecticides	Original	+5 PB		+5 MGK 264	
	LC_{50} (slope)	LC_{50} (slope)	S. R.	LC_{50} (slope)	S. R.
Susceptible strain					
Decamethrin	26.2 (0.62)	15.0 (1.02)	1.75	14.2 (0.57)	1.85
Fenvalerate	48.4 (0.68)	40.6 (1.11)	1.19	40.4 (1.08)	1.20
Permethrin	98.0 (1.18)	44.1 (1.18)	2.22	45.8 (0.50)	1.14
Resistant strain					
Decamethrin	214.7 (0.46)	35.6 (0.99)	6.00	64.2 (0.61)	3.34
Fenvalerate	756.9 (0.61)	45.9 (0.96)	16.50	58.2 (0.77)	12.99
Permethrin	353.2 (0.85)	44.8 (1.43)	7.88	168.9 (1.24)	2.09

The test results of carbamates (Table 5) demonstrated that the MFOs of DBM have different substrate-preference for carbaryl and carbofuran. It was noticed that the original LC_{50} difference between carbaryl and carbofuran in the susceptible strain were enormous, i.e., 16,426 vs. 150ppm. After the pb was incorperated, the LC_{50} difference was narrowed to 350.7 vs. 106.7ppm, or only 3 fold. The synergistic ratios were 46.83 vs. 1.40, which indicated that the carbaryl can be effectively synergized by pb but not for carbofuran. It is not probable that pb can discriminate different carbamates, but rather due to the MFOs with greater substrate-affinity for carbaryl than carbofuran.

Table 5. The effects of piperionyl butoxide and MGK 264 on the DBM resistance to carbamates. (LC_{50} in ppm)

Insecticides	Original	+5 PB		+5 MGK264	
	LC_{50} (slope)	LC_{50} (slope)	S. R.	LC_{50} (slope)	S. R.
Susceptible strain					
Carbaryl	16,426.4 (1.05)	350.7 (1.15)	46.83	—	—
Carbofuran	150.3 (0.57)	106.7 (1.20)	1.40	63.8 (0.95)	2.40
Resistant strain					
Carbaryl	21,818.0 (0.82)	544.2 (1.24)	40.09	1,067.2 (0.98)	20.4
Carbofuran	570.0 (0.67)	105.4 (0.97)	5.40	149.3 (1.04)	3.8

The carbofuran-preferred MFOs were better developed in the resistant DBM, and the LC_{50} was 570.0ppm or 3.8 fold of that for the susceptible DBM. The MFOs activity

probably was induced, and subjected to the inhibitory action of pb. The pb had successfully restored the carbofuran-sensitivity in both resistant and susceptible DBMs as their LC_{50}s were 105.4 and 106.7ppm, respectively.

3. The MFO specificity study: in combination with pb or pe, four carbamates were tested on both the susceptible and the highly resistant Taichung strain (Table 6).

Table 6. The selectivity of the DBM's oxidative detoxication towards different carbamates. (LD_{50} in ng per insect)

Insecticides	Original	+5 PB		+2 PE	
	LD_{50} (slope)	LD_{50} (slope)	S. R.	LD_{50} (slope)	S. R.
Susceptible strain					
Carbaryl	4,656.8 (0.54)	190.1 (1.04)	24.5	166.1 (0.59)	28.0
Methomyl	1,900.5 (0.82)	172.6 (0.98)	11.0	102.5 (1.36)	18.6
Propoxur	2,160.8 (0.61)	205.3 (0.82)	10.5	142.9 (0.91)	15.1
Carbofuran	75.2 (0.44)	26.7 (0.84)	2.8	17.1 (1.02)	4.4
Highly resistant strain					
Carbaryl	— *	342.2 (0.89)	—**	465.5 (1.19)	—**
Methomyl	19,674.4 (0.43)	381.4 (1.41)	51.6	262.4 (1.81)	75.0
Propoxur	— *	430.2 (1.14)	—**	285.1 (1.78)	—**
Carbofuran	263.7 (0.58)	111.2 (0.51)	2.4	66.1 (0.97)	4.0

*concentration is too high to be determined by topical application method.
**can not be calculated.

In the susceptible strain, the MFOs had the substrate preferences in the order of carbaryl>propoxur>methomyl>>carbofuran. The affinities for the first three carbamates were very similar as the differences among them were no more than 3 fold. The affinity for carbofuran is 25.3, 28.7, and 61.9 fold lower than methomyl, propoxur, and carbaryl, respectively. By adding the synergists, all three ineffective carbamates had recovered their insecticidal potencies against the DBM as the LD_{50}s shown in Table 6 were effectively lowered 10-24 fold. Again, carbofuran was not synergized so much as other carbamates; this discrepancy indicated that limited MFOs activity toward carbofuran is existing in the DBM. Similar synergistic ratios were recorded in both the susceptible and the resistant DBM, except the synergistic ratios increased 4-5 fold for methomyl in the resistant DBM.

Due to the limitation of testing method and the high resistance, the toxicity of carbaryl and propoxur to the highly resistant Taichung DBM could not be measured; the LD_{50} of methomyl was measured at 19,674.4ng/insect, which was 10 fold higher than that of the susceptible DBM. Once the synergist was added, even the highly resistant DBM

became susceptible to all carbamates as their LD_{50}s were lowered to 342.2, 381.4, and 430.2ng/insect for carbaryl, methomyl, and propoxur, respectively. The result of pe test is very similar to that of pb except the synergistic effect of pe is stronger.

Discussion

Important information concerning the DBM resistance to insecticides was gathered in this investigation and would be helpful in clarifying how and why the DBM is responded to different insecticides.

The resistance declining rate in the short span of generations (F_5)is important because the DBMs in vegetable field hardly have a chance to escape from the insecticide contact in a prolonged period. Hence in practical sense, the results of F_5 in Table 2 are more useful in predicting the resistance developing trend for an insecticide than that of F_8 or F_{11}.

In the OP insecticides, mevinphos, phenthoate, and methidathion are the compounds with the fastest declining rates in resistance; coincidentally,these three compounds had met the slightest resistance in the field. On the other hand, methamidophos, mephosfolan, and diethquinalphion had the slowest rates and also met with enormous resistance [18]. Due to the resistance declining order matched the status of resistance in the field, we suspected that the natural declining rate of resistance for OP insecticides is one of the main reasons why some OP insecticides still retain their DBM control efficacies despite the long period of field usage.

Our test results had showed that the OP-resistance was not reduced by pb, in other word, was not related to the MFOs action. The induction and reduction of MFOs activity usually are rapid, and the independence of OP-resistance from MFOs may be the explanation why OP-resistance can not reach the high level for both carbamates and synthetic pyrethroids. For example, the OP-resistance was ranged 2.87-9.26 fold(Table2), while 40-200 fold resistances were easily recorded for carbaryl and synthetic pyrethroids. However, the OP-resistance of DBM may associate with other factors such as esterases, hydrolases, glutathione-S-transferases or acetylcholinesterase-insensitivity [8,9,16,22] etc.

Liu et al. had reported that pb could successfully synergize fenvalerate, decamethrin, and permethrin; their synergistic ratios were ranged 1.8-4.3 for the susceptible FS strain and 5.7-15.4 for the resistant BC strain [19]. In contrast, we have found the synergistic ratios were 1.2-2.2 for the susceptible I-lan strain and 6.0-16.5 for the F_5 generation of resistant Taipei strain. In both studies, fenvalerate is the one with both the highest resistance and synergistic ratio as compared to other synthetic pyrethroids. Whether the substrate-preference is existed in the MFOs for different synthetic pyrethroids is an interesting matter for further investigation. The low synergistic ratios observed in I-lan strain have confirmed a long suspected fact that the DBM in I-lan area has not developed the MFOs for detoxicating synthetic pyrethroids yet.

Although the SP-resistance declined in a very fast pace, the remained resistance is still too high for the practical use of synthetic pyrethroids.

We also observed a portion of SP-resistance that was not eliminated by pb and hence was not related to the action of MFOs. The magnitude of this pb-insensitive resistance is

ranged 3-9 in resistance ratios for different compounds and its origin is not clear. Liu et al. recorded a 2 fold synergism by esterase inhibitors in synthetic pyrethroids while with the highest synergistic effect for permethrin. The finding is more obvious in the FS strain than in the resistant BC strain indicating that the esterase for detoxication may be induced by either synthetic pyrethroids [19] or other factors. Riskallah had reported the increase in esterase activity was closely associated with the development of SP-resistance in *Spodoptera littoralis* [24]. Is the pb insensitive SP-resistance due to the esterase activity? Can this SP-resistance exert cross effect in detoxicating OP insecticides? However, minor cross resistance had been observed in our previous study [10].

Another important part of this study is that DBM's MFOs do not detoxicate carbofuran as effectively as other carbamates, unless the carbofuran-preferred MFO was first induced. We had found that carbofuran acted differently from other carbamates in their corresponding toxicities on DBMs, and the carbofuran resistance also acted independently from other insecticides [11]. Current investigation result has reconfirmed those findings. Most carbamates are not effective DBM control agents even for the susceptible I-lan strain [11]. Coincide with our finding, Liu et al. had tested 15 insecticides on their highly susceptible FS strain, and the three least effective insecticides on the list were carbaryl, propoxur, and methomyl [20]. Brattsten and Metcalf had reported that the MFOs activity is not the same in natural insect populaion [4,5]; the houseflies, *Musca domestica*, served as a good example that even in the susceptible strain, the naturally occurring MFOs is enough to immune the insects from the lethal action of carbaryl [13,26]. It is doubtful that how important the induced MFO is responsible for the carbaryl-resistance (or carbaryl-tolerance) in DBM, compared to the naturally occurring MFOs which also exert its cross resistance to propoxur and methomyl. The MFOs is definitely the most important single detoxication mechanism in DBM for both carbamates and synthetic pyrethroids, and all above mentioned MFOs activities ares ubjected to the inhibitory action of pb, MGK 264, and pe.

Base upon the findings of (1) the I-lan strain is susceptible to both synthetic pyrethroids and carbofuran but not three other carbamates; (2) the Taipei strain is resistant to carbaryl and synthetic pyrethroids but only mild resistant to carbofuran; (3) the SP-resistance and carbofuran-resistance have different declining rates; (4) no corelation can be found between SP-resistance and carbofuran-resistance in field survey [10]; (5) the Taichung strain is extremely insensitive to most carbamates but only mild resistant to carbofuran; and (6) in the carbofuran selection, the susceptible I-lan strain rapidly developed 36 fold resistance in 8 generations and with no sign of cross resistance to other insecticides [11], we can tentatively concluded that there are three catagories of MFOs in DBM for detoxicating the insecticides: one for the synthetic pyrethroids, one for carbofuran, and another for other carbamates. It is unlikely that the cross resistance is existed among these three catagories of MFOs in DBMs.

References

1. Abdel-Aal, Y. A. I. and D. M. Soderlund. 1980. Pyrethroid-hydrolyzing esterases in southern armyworm larvae: tissue distribution, kinetic properties, and selective inhibition. Pestic. Biochem. Physiol. 14 : 282-28?.
2. Agosin, M. and A. S. Perry. 1974. In"The Physiology of Insecta" (M. Rockstein, ed.) , 2nd ed., Vol. V, pp. 580, Chapter 10. Microsomal Mixed-function Oixdase. Academic Press, New York and London.
3. Barnes, J. R. and J. Fellig. 1969. Synergism of carbamate insecticides by phenyl 2-propynyl esters. J. Econ. Entomol. 62 : 86-89.
4. Brattsten, L. B. and R. L. Metcalf. 1970. The synergistic ratio of carbaryl with piperonyl butoxide as an indicator of the distribution of multifunction oxidases in the insecta. J. Econ. Entomol. 63 : 101-104.
5. Brattsten, L. B. and R. L. Metcalf. 1973. Synergism of carbaryl toxicity in natural insect populations. J. Econ. Entomol. 66 : 1347-1348.
6. Casida, J. E. 1970. Mixed-function oxidase involvement in the biochemistry of insecticide synergists. J. Agr. Food Chem. 18 : 753-772.
7. Cheng, E. Y. 1981. Insecticide resistance study in *Plutella xylostella* (L.) II. A general survey (1980-81). J. Agric. Res. China 30 : 285-293.
8. Cheng, E. Y., T. M. Chou, and C. H. Kao. 1983. Insecticide resistance study in *Plutella xylostella* (L.) IV. The activities of glutathione-S-transferase in the organophosphorus-resistant strains. J. Agric. Res. China 32 : 373-378.
9. Cheng, E. Y., T. M. Chou, and C. H. Kao. 1984. Insecticide resistance study in *Plutella xylostella* (L.) V. The induction, cross resistance and glutathione-S-transferase in relation to mevinphos-resistance. J. Agric. Res. China 33 : 73-80.
10. Cheng, E. Y., T. M. Chou, and C. H. Kao. 1985. Insecticide resistance study in *Plutella xylostella* (L.) VI. An experimental analysis of organophosphorus and synthetic pyrethroid resistances. J. Agric. Res. China 34 : 96-104.
11. Chou, T. M. and E. Y. Cheng. 1983. Insecticide resistance study in *Plutella xylostella* (L.) III. The insecticide susceptibilities and resistance response of a native susceptible strain. J. Agric. Res. China 32 : 146-154.
12. Eagleson, C. 1940. Oil synergists for insecticides. U. S. Patent 2,702,145. May 4.
13. Eldefrawi, M. E., R. Miskus, and V. Sutcher. 1960. Methylenedioxyphenyl derivatives as synergists for carbamate insecticides on susceptible, DDT-, and parathion-resistant house flies. J. Econ. Entomol. 53 : 231-234.
14. Fellig, J., J. R. Barnes, A. I. Rachlin, J. P. O'Brien, and A. Focella. 1970. Substituted phenyl 2-propynyl ethers as carbamate synergists. J. Agr. Food Chem. 18 : 87-89.
15. Finney, D. J. 1971. Probit analysis. University Press, Cambridge.
16. Hassall, K. A. 1982. The chemistry of pesticides, their metabolism, mode of action and uses in crop protection. The MacMillan Press Ltd., London.
17. Hoffman, R. A., T. L. Hopkins, and A. W. Lindquist. 1954. Tests with pyrethrum synergists combined with some organic phosphorus compounds against DDT-resistant flies. J. Econ. Entomol. 47 : 72-76.
18. Kao, C. H., E. Y. Cheng, and D. F. Lin. 1986. Insecticide resistance study in *Plutella xylostella* (L.) VII. Action patterns of insecticides and the resistance impacts. J. Agric. Res. China

35 : 107-117.

19. Liu, M. Y., J. S. Chen, and C. N. Sun. 1984. Synergism of pyrethroids by several compounds in larvae of the diamondback moth (Lepidoptera: Plutellidae). J. Econ. Entomol. 77 : 851-856.
20. Liu, M. Y., Y. J. Tzeng, and C. N. Sun. 1982. Insecticide resistance in the diamondback moth. J. Econ. Entomol. 75 : 153-155.
21. Moorefield, H. H. 1958. Synergism of the carbamate insecticide. Contr. Boyce Thompson Inst. 19 : 501-508.
22. Oppenoorth, F. J. 1984. Biochemistry of insecticide resistance. Pestic. Biochem. Physiol. 22 : 187-193.
23. Pearson, A. M. 1935. The role of pine oil in cattle fly sprays. Delaware Agric. Expt. Bull. 196.
24. Riskallah, M. R. 1983. Esterases and resistance to synthetic pyrethroids in the Egyptian cotton leafworm. Pestic. Biochem. Physiol. 19 : 184-189.
25. Sacher, R. M., R. L. Metcalf, and T. R. Fukuto. 1968. Propynyl naphthyl ethers as selective carbamate synergists. J. Agr. Food. Chem. 16 : 779-786.
26. Sacher, R. M., R. L. Metcalf, E. R. Metcalf, and T. R. Fukuto. 1971. Structure activity relationships of methylenedioxynaphthyl derivatives as synergists of carbaryl in house flies. J. Econ. Entomol. 64 : 1011-1014.
27. Terriere, L. C. and S. J. Yu. 1979. Cytochrome P-450 in insects. 2. Multiple forms in the flesh fly (*Sarcophaga bullata*, Parker) and the blow fly (*Phormia regina* (Meigen)). Pestic. Biochem. Physiol. 12 : 249-256.
28. Yu, S. J. and L. C. Terriere. 1979. Cytochrome P-450 in insects. 1. Differences in the forms present in insecticide resistant and susceptible house flies. Pestic. Biochem. Physiol. 12 : 239-248.

小菜蛾抗藥性之研究

Ⅷ 幼蟲體內氧化解毒酵素之專屬性[1]

鄭　允　高靜華　林端方　蔡湯瓊[2]

摘　要

本研究之目的在於測定小菜蛾幼蟲體內氧化解毒酵素對各類殺蟲劑之專屬性，以探討是否與抗藥性有關。以不同地區採得之感性及抗性小菜蛾爲材料，除比較不同殺蟲劑之感度，折算成抗性比率外；並觀察抗性小菜蛾不再接觸農藥後，抗藥性下降的情形。另一方面，利用氧化解毒酵素之抑制劑(就毒效而言爲協力劑，如 piperionyl butoxide)，測定對各種殺蟲劑的協力程度，以決定氧化酵素對該殺蟲劑抗性所扮演之角色及其比重。結果發現有機磷類殺蟲劑受氧化解毒酵素影響甚小，與其抗藥性幾乎無關；但在試驗中發現小菜蛾幼蟲對美文松、賽達松及滅大松之抗性消退甚快，而對拜裕松、達馬松及美福松之抗性消退極慢，此一現象正好與該等藥劑在田間小菜蛾之抗性高低相吻合。影響抗藥性消退速率的原因，目前尚不淸楚，小菜蛾對有機磷之抗性程度一向不及對氨基甲酸鹽或合成除蟲菊精劑，可能是與氧化解毒酵素無關之故。就合成除蟲菊精類殺蟲劑而言，3種供試藥劑幾乎有相同之抗性消退速率，經過6代以後，抗性消退至原來抗性之1/10；雖然其抗性消退速率甚快，但在田間之抗性極高，可能係氧化解毒酵素之誘發與消退均極爲迅速。使用協力劑測定時發現，氧化解毒酵素爲合成除蟲菊精類殺蟲劑抗藥性之主要機制，使用協力劑可使抗性小菜蛾恢復對除蟲菊精類殺蟲劑之敏感度，數種合成除蟲菊精類在此試驗中之表現均極一致，但仍有少部份之除蟲菊精抗性與氧化解毒酵素無關，是否來自酯酶，則有待進一步之求證。氨基甲酸鹽類殺蟲劑之測定結果發現：加保扶之毒理作用較特殊，與加保利、安丹及納乃得有差別。感性品系小菜蛾之氧化解毒酵素可以解去加保利、安丹及納乃得之毒性作用，然却無法消除合成除蟲菊精之致毒能力，亦無法對加保扶發生解毒作用，可能爲氧化解毒酵素之另一支。小菜蛾亦能對加保扶產生極高之抗藥性，且該抗藥性與其他氨基甲酸鹽類或合成除蟲菊精劑均無交互抗性，但 piperionyl butoxide 可以消除此一抗藥能力。由此判斷，小菜蛾之氧化解毒系統可能分別對（1）合成除蟲菊精類，（2）加保扶及（3）其他三個氨基甲酸鹽類，形成三個獨立的解毒系統，而其間可能不具有交互抗性關係。

1.臺灣省農業試驗所　研究報告第 1295 號。本計畫承國科會及農委會補助（計畫編號 NSC-75-0409-B055-10及74農建—4.1—產植—147 (03)；簡淑貝、盧燕鈴協助工作，僅此一併致謝。

2.本所應用動物系研究員、研究助理、計畫助理及計畫助理。臺灣省　臺中縣　霧峰鄉。